REMARKS

Claims 1, 3-5, 7, 8, 10-23 are pending in this application. Claims 2, 6 and 9 have been cancelled. Claims 10-23 have been withdrawn. Claims 1, 3-5, 7 and 8 stand rejected under §§ 102(b), 103(a) and/or 112, second paragraph. Claims 5 and 7 have been amended,

In an effort to simplify prosecution and expedite allowance, Applicants have amended claims 5 and 7 to clarify the claims and to overcome the rejections. Support for amendments to claim 7 may be found in paragraphs [0020] and [0034] of the printed U.S. Patent Publication 2005/0250167, and in the claims themselves, for example. It is believed that no new matter has been added.

Rejection under Section 102(b)

The Examiner rejected claims 1, 3-5, 7 and 8 under 35 U.S.C. § 102(b) as being inherently anticipated by Brown (US Patent No. 6,322, 962), Joly et al. (US 6,342,495), or Cravatt et al. (US 2002/0040275 (in view of Toker et al., *J. Biol. Chem.*, (2000) 275:8271-4). The Examiner argued that Brown, Joly and Cravatt teach compositions comprising membrane fractions of HEK 293 cells. The Examiner did not specify which property of the claimed invention is inherently disclosed in Toker et al., but seems to have argued that the PKB Ser 473 kinase activity in PBK/Akt is inherently disclosed because Toker et al. shows that the PBK/Akt, when associated with cellular proteins, has PKB Ser 473 kinase activity.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). Although extrinsic evidence such as a second reference may be used to show an inherent characteristic within the teaching of the primary reference, "[the second reference] must make clear that the missing descriptive matter is necessarily present in the thing described in the [primary] reference, and that it would be so recognized by persons of ordinary skill." *Continental Can Co. USA v. Monsanto Co.*, 948 F.2d 1264, 1268 (Fed. Cir. 1991).

Brown, Jolly and Cravatt fail to anticipate the claims and are only similar in that they share the use of HEK 293 cells, which are commonly used cell lines in cell biology experiments. Turning to Brown, Brown merely discloses a general preparation of a cell-free

extract comprising a Site-1 protease, which has nothing to do with the protein Kinase B pathway. The additional reference of Jolly, simply discloses a membrane-free extract of 293 cells expressing a human peripheral benzodiazepine receptor, which again has no relevance to the compositions, extracts, and complexes disclosed by the Applicant. Cravatt merely discloses a membrane-free extract of 293 cells expressing an acylpeptide hydrolase, and fails for similar reasons. The only common factor between these references and the Applicant's invention is the use of HEK293 cells, which are human embryonic kidney 293 cells that are cells used in cell biology experiments. None of the references supplement Toker and nowhere disclose a cell-free composition comprising a "complex which has a *PKB Ser 473 activity* and an apparent molecular weight of *450-650 kDA.*"

Toker fails to anticipate for several reasons, because subsequent articles showed that PKB/Akt does not autophosphorylate and thus do not show PKB Ser473 activity, thereby showing that the kinase complex disclosed by the Applicants is distinct from PKB/Akt. Although Toker et al. shows that PBK/Akt has PKB Ser 473 kinase activity, the accuracy of this has been questioned by those skilled in the art. As cited in Applicants' previous response, Hill et al., *J. Biol. Chem.* (2001) 276(28):25643-6 shows that staurosporine, a broad-specificity kinase inhibitor, potently inhibited PDK1 activity without affecting Ser 473 phosphorylation, which suggests that phosphorylation of Ser473 of PKB is via a distinct Ser-473 kinase and not through PDK1 or PKB activity. In fact, Alex Toker himself acknowledges such skepticism in a later article titled "Akt signaling: A Damaging Interaction Makes Good", *Trends in Biochemical Sciences*, (2008) 33(8):356-359 ("Second Toker Article")[1] where he recognizes that the "[i]nitial findings that PDK-1, the integrin-linked kinase or Akt itself was the Ser473 kinase [ ] were met with considerable skepticism" and acknowledges a study by the Sabatini group which shows the relevant Ser 473 kinase. *Id.* at p.358. These two articles, therefore, suggest that PKB/Akt does not autophosphorylate. This conclusion (i.e., that PKB/Akt does not autophosphorylate) is also

[1] It is noted that the Second Toker Reference was cited to support an argument against that raised by the Examiner during the final office action and to provide information about the state of the art after the filing date of the current application and therefore should not be construed to be an admission that the information cited is material to patentability or prior art.

well known/accepted in the art. As such, the Examiner has not established that PBK/Akt has PKB Ser473 kinase activity.

Furthermore, the kinase complex is distinct from PKB/AKt as it has a different molecular weight. Even if the PBK/Akt arguably has PKB Ser 473 kinase activity, the prior art cited does not disclose, explicitly or inherently, the molecular weight limitation of the claims. Nothing in the prior art therefore suggests a complex having a molecular weight of 450-650kDa. In fact, prior to the filing of the current application, it is not known that Ser473 kinase operates via a multi-subunit/protein complex as disclosed in the currently claimed invention. This discovery, however, is consistent with the later finding by the Sabatini group as discussed in the Second Toker Article, which shows that the relevant Ser 473 kinase, in fact, operates as complex.

Moreover, the Toker reference teaches away from the claims and cannot disclose the kinase complex, either explicitly or inherently. Toker states that Ser-473 phosphorylation requires the activity of Akt (autophosphorylation) and "cannot result from contaminating kinases or from phosphorylation by PDK-1, as suggested recently." (paragraph 1, pg. 8274.) This precludes the possibility of a kinase complex having an apparent molecular weight of 450-650 kDa, as recited in the claim. Therefore, the claims are not anticipated by Toker.

Furthermore, the methods to study the claimed kinase complex are different from those disclosed by Toker. Toker *et al.* uses and discloses a pure Akt which has a MW of 62-64 kDA. (See pg. 8273, 2nd paragraph, under Results and Discussion.) Pg. 8273 pointed out by the Examiner does not disclose a protein complex that has a MW of 450-650 kDa as claimed by the Applicants. The reference at the cited page, at most, discloses a wild-type and a mutant AKT in complex with anti-HA antibodies. On the other hand, the claimed complex, was isolated using a very specific method. (See paragraphs [0010], [0012]-[0017], and specifically, paragraph [0014], wherein an attempt to over-purify, e.g., using ion exchange chromatography resulted in loss of the activity of the complex.) It is respectfully submitted that Akt disclosed in Toker isolated simply using anti-HA antibodies is not the protein complex disclosed by the Applicants.

Furthermore, the specification itself identifies numerous reasons for distinguishing the kinase complex disclosed by the applicants from PDK-1 and autophosphorylation.

First, location distinguishes the kinase complex from PKB and PDK-1. The kinase complex was substantially enriched in the plasma membrane fraction, unlike PKB and PDK-1, which are mainly located in the cytosolic fraction. For example, paragraph 102 of the published application notes that the total kinase complex was substantially more in the plasma membrane fraction and specific activity for Ser 473 phosphorylation was found to be highly enriched in the plasma membrane fraction of HEK 293 cells. In contrast, PKB and PDK1 are mainly located in the cytosolic fraction of unstimulated cells, as disclosed by the applicant in the same paragraph. Indeed, the Anderson reference, submitted with this response, shows that PDK-1, generally in the cytsol, translocates to the plasma membrane under certain conditions, such as PDGF stimulation. (See pg. 689, first paragraph.) This reaffirms the differences between the kinase complex identified by the Applicants and the PDK-1. Therefore, the claims are not anticipated.

In addition, numerous paragraphs affirm that the Ser473 kinase complex is associated with the plasma membrane and is not an integral membrane protein. For example, paragraph 51 discloses that extraction of the plasma membrane fraction with a high ionic strength buffer releases the Ser473 kinase from the lipid bilayer, thus showing that this kinase complex is not an integral membrane protein.

Secondly, the kinase complex operates through a distinct mechanism from PDK-1 and thus, they are different. For example, PDK-1 kinase activity is dependent on PI3-kinase signaling, as identified by the second Toker article. In paragraph 103, the applicants test if the membrane associated Ser 473 kinase activity was dependent on PI3-kinase signaling. Insulin and pervanadate both induced phosphorylation of Thr308. However, neither stimuli increased Ser 473 kinase complex activity above basal levels, thus showing that the Ser473 kinase complex operates through a distinct mechanism from PDK-1. Treatment with a PI3 kinase inhibitor did not increase Ser 473 kinase activity in the cytosolic fraction, thus showing that Ser 473 is associated with the plasma membrane, again distinguishing from the cytosolic-dominant PDK-1. Furthermore, paragraph 114 shows that further attempts to purify the Ser 473 kinase complex resulted in a loss of activity, thus affirming that this disclosed kinase is a kinase complex. Thus, the Applicants distinguish PDK-1 from the kinase complex disclosed by the applicants.

Also, the kinase complex disclosed by the Applicants is distinct from PKB because PKB isoforms could be phosphorylated under conditions where PKB could not. For

example, the applicants teach that phosphorylation of Ser 473 does not occur through autophosporylation but through the disclosed kinase complex. Paragraph 102 discloses that PKB does not autophosphorylate on Ser 473, under conditions when even the partially purified Ser473 kinase preparation could phosophorylate various PKB isoforms, including a kinase inactive mutant of PKB-alpha. In addition, Figure 2 (corresponding to Example 3) show that the PKB Ser 473 kinase activity eluted in fractions 17-20, and phosphorylation of the recombinant PKB protein was measured using phospho specific-Ser 473 specific antibodies. Furthermore, using a highly purified crystallization grade PKB, no autophosphorylation of PKB could be seen and thus phosphorylation of Ser 473 was dependent upon addition of the partially purified Ser 473 kinase. Furthermore, Example 5 discloses that PKB isoforms and mutants were not phosphorylated on Ser473 when incubated in kinase reaction buffer, thus showing that PKB does not autophosphorylate on Ser 473. Therefore, the kinase complex is distinguished from the references.

The kinase complex disclosed by the Applicants can phosphorylate PKB isoforms and is thus distinct from the autophosphorylation mechanism identified by Toker. For example, these PKB isoforms when incubated with partially purified Ser473 kinase, with the exception of a PKB with a mutated Ser473 site, were all phosphorylated on Ser473. Even the ATP binding site mutant K179A and the activation loop phosphorylation site mutant T308A (PKB mutants) were not impaired in their ability to be phosphorylated on Ser473, thus showing that PKB kinase activity is not required for maximal Ser473 kinase activity. (See paragraph [0116], for example.)

The kinase complex disclosed by the Applicants can thus phosphorylate the activation loop mutants where Toker would affirm that phosphorylation could not occur. In fact, Toker teaches an opposite mechanism from those disclosed by the Applicants and states that phosphorylation at the activation loop triggers autophosphorylation of Ser473.(pg. 8273, last paragraph.) However, the activation loop mutant T308 disclosed in the Applicant's examples *maintained phosphorylation*, thus showing that the phosphorylation occurs through the Ser 473 kinase disclosed by the Applicants, and does not operate through autophosphorylation. If the autophosphorylation mechanism identified by Toker was the mechanism, then the activation loop mutant would not have been phosphorylated. Therefore, the applicants' experimental evidence in

the specification affirms that the Ser473 kinase is distinct from autophosphorylation identified by Toker.

Applicants respectfully submit that the prior art references do not explicitly or inherently disclose every element of the claimed invention. Withdrawal of the rejections under 35 U.S.C. § 102 is earnestly requested.

Rejection under 35 U.S.C.§ 112, Second Paragraph

The Examiner rejected claim 7 under Section 112, second paragraph for failing to point out and distinctly claim the subject matter for which applicant regards as his invention. Without agreeing to the accuracy of the Examiner's conclusion, Applicants hereby amend claim 7 to clarify them. For example, paragraphs [0020] and [0034] identify what would constitute measurable activity. The amendments thus overcome the Examiner's rejection. Applicants therefore earnestly request entry of the amendments.

CONCLUSION

Applicant respectfully submits that the claims are now in condition for allowance and notification to that effect is earnestly requested.

Respectfully submitted,

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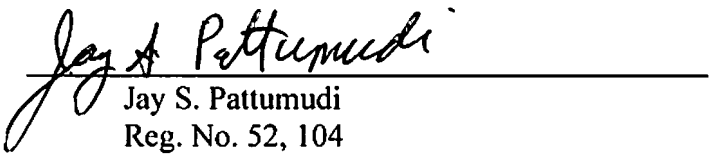

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